Molecular simulation of protein adsorption and conformation at gas-liquid, liquid–liquid and solid–liquid interfaces

Molecular Simulation of Protein Adsorption and Conformation at Gas-Liquid, Liquid-Liquid and Solid-Liquid Interfaces

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Abstract

The adsorption of proteins at surfaces and interfaces is important in a wide range of industries. Understanding and controlling the conformation of adsorbed proteins at surfaces is critical to stability and function in many technological applications including foods and biomedical testing kits or sensors. Studying adsorbed protein conformation is difficult experimentally and so over the past few decades researchers have turned to computer simulation methods to give information at the atomic level on this important area. In this review we summarize some of the significant simulation work over the past four years at both fluid (liquid-liquid and gas-liquid interfaces) and solid-liquid interfaces. Of particular significance is the work on surfactant proteins such as fungal hydrophobins, ranspumin-2 from the túngara frog and the bacteria protein BslA. These have evolved unique structures impart very high surface-active properties to the molecules. A highlight is the elucidation of the clam-shell unhinging mechanism of ranspumin-2 adsorption to the gas-liquid interface that is responsible for its adsorption to and stabilization of the air bubbles in túngara frog foam nests.

Keywords: Molecular dynamics; protein adsorption; fluid interfaces; solid-water interfaces

1. Introduction

The adsorption of proteins to interfaces, both fluid and solid, is an important phenomenon in many technological processes across a wide range of industry sectors. For example, in foods proteins are used to form oil-water and air-water fluid interfaces in emulsion and foam products, and provide stability against coalescence and disproportionation in food and beverage foams [1]. Proteins will also adsorb to a solid surface during food processing and form a foulant layer that can reduce efficiency of heat transfer, or can support microbial growth [2]. Protein adsorption to solid surfaces is also important in biomedicine. Protein adsorption to surgical instruments, and how these can be cleaned efficiently or adsorption prevented in the first place, is a significant concern [3]. The promotion of protein adsorption to surgical implants (such as joint replacements) is essential to facilitate integration with bone and tissues in the body [4]. Similarly, many modern medical (and other) diagnostic tests are based around the adsorption of a sensing molecule (often an enzyme) to a silicon chip (so-called lab on a chip devices) [5]. With these, maintaining the tertiary structure of the adsorbed protein is essential, as this structure enables the biological activity required for sensing.

With all of these applications, an understanding of the changes in the conformation of proteins as they adsorb to interface is key to controlling the conformational stability of the protein, which is a key factor in functionality. Computer modelling is finding an increasing role in these investigations, since it is able to give detailed molecular level information at a scale that is difficult to study experimentally. In this review, we will cover the advances in molecular modelling of protein adsorption at fluid and solid interfaces over the past 3-4 years, whilst also including some earlier references where

relevant. For detailed information on earlier modelling efforts reference should be made to previous reviews of the subject matter [6].

2. Modelling of Protein Adsorption at Fluid Interface

The majority of simulation studies on protein adsorption have been on compact, globular proteins, with surprisingly few studies on unstructured intrinsically disordered proteins. This may be because globular proteins display a richer conformational behaviour, with perturbations in these easily followed using molecular dynamics (MD) simulation. McGrath and co-workers [7, 8] elucidated the adsorption mechanisms for the bovine whey protein β-lactoglobulin (β-lac) at an oil-water interface (decane) and the effect of varying the oil phase hydrophilicity on adsorbed conformation. Various starting orientations of the β-lac molecule were modelled, but in each case, the orientation upon adsorption and the amino acid residues in contact with the surface were similar. Lysine residues in positions 14 and 77 formed initial contacts with the surface in nearly all simulations. The β-lac molecule did not form a stable adsorbed conformation immediately upon contacting the surface, but diffused across the surface before finally adsorbing. As adsorption progressed, residues in the N-terminal region and residues 70-123 where there are significant proportions of hydrophobic amino acids show a high probability of contacting the surface. The conformation of β-lac does not change on contact with the surface, but unfolds with time after a stable adsorbed state forms, eventually allowing around 80-90 residues to interact with the decane layer. Unfolding of the tertiary fold of the β-lac is not accompanied by loss of secondary structure, suggesting the latter is highly stable.

There is evidence that the mechanism of adsorption varies for different interfaces. After adsorption, the orientation of β-lac at the triolein interface varies little, whilst fluctuations occur at the decane and octanol surfaces. Additionally, the mechanism of adsorption differs. At the decane surface, a small number of interactions are made initially, followed by a rapid increase as the adsorption process completes. β-lac adsorbing to an octanol surface also starts with a small number of interactions, but is then followed by sequences of rapid, slow and no increase in interactions. At the triolein interface, β-lac has a different behaviour where there is a gradual increase in surface interaction over 10's of ns after initial adsorption.

Cheung [9*, 10*] has used replica exchange MD to probe the adsorption at an octane-water interface of myoglobin peptides, lysozyme and α-lactalbumin, demonstrating the importance of intra-protein interactions in adsorbed conformations. These proteins display differing structural stability – myoglobin lacks disulphide bonds unlike lysozyme and α-lactalbumin, and is more flexible at the interface, and so these make ideal model proteins for interfacial studies. Two myoglobin peptides comprising amino acids 1-55 and 56-131 have been simulated [10*] and the results compared to experimental information on the adsorbed conformation of the two [11, 12]. The peptides share structural similarities, such as a similar proportion of hydrophobic amino acid side chains and α-helical character, but display differing adsorption behaviour. Multiple simulation runs suggested that the more flexible 1-55 peptide adopts three conformations at the interface, one compact and two extended, with one of the extended conformations preferred (Figure 1), with the two extended states corresponding to differing orientations of helices at the interface. Peptide 56-131 on the other hand prefers a compact native-like conformation at the interface. Interestingly, the two peptides also show differing emulsifying and foaming activity,

with peptide 1-55 being a good emulsifier and foamer whilst peptide 56-131 is poor at emulsification and foaming [11] suggesting link between adsorbed conformation and these properties.

The same group [9*] has shown that lysozyme and α-lactalbumin also adopt a range of conformations at the octane-water interface, with compact globular states being more common for both proteins, and extended states rarer. Differences in adsorption are observed, despite the similarity of the two proteins, in that lysozyme adsorption appears to be non-specific, whereas α-lactalbumin adsorption seems to be mediated through two amphipathic α-helices. To understand further the factors controlling the interfacial conformations, the free energy differences between native and adsorbed states was calculated. This was decomposed into terms described protein-interface interactions ($\Delta F_{p\text{-}i}$), protein-protein intra molecular interactions ($\Delta F_{p\text{-}p}$) and a partition term (ΔF_{part}). The balance between these terms determines the degree of unfolding, with $\Delta F_{p\text{-}I}$ favouring extended conformations and $\Delta F_{p\text{-}p}$ compact conformations. Lysozyme and α-lactalbumin were found predominantly in compact conformations, indicating that intra-molecular interactions dominate over protein-surface interactions. A further difference in the behaviour of the two proteins is in the interactions between the protein and the octane phase. Protein-oil interaction was more favourable in the extended state of α-lactalbumin compared to the compact state, whilst this interaction was not significantly different for lysozyme between the two states. This would suggest that the hydrophobic phase influence on protein conformation would differ between proteins. The behaviour of lysozyme and α-lactalbumin is in contrast to myoglobin where extended conformations are favoured. This reflects weaker intra-peptide interactions in myoglobin, mainly from the lack of disulphide bonds in the structure.

Another protein whose adsorption to fluid interfaces has been widely studied is barley lipid transfer protein (LTP). LTP is one of main proteins responsible for the foaming properties of beer. In its native state LTP is stabilized by four disulphide bonds that impart high conformational stability [13]. During the wort boiling stage of the brewing process LTP is partially denatured, glycosylated and disulphide bonds are broken [14]. These changes improve the foaming ability. Early studies of LTP adsorption at air-water and decane-water interface [15-17] highlighted the different conformations adopted at the two interfaces. LTP adsorbed to the air-water interface shows only minor perturbations from the native tertiary conformation. At the decane interface, on the other hand the LTP molecule penetrates into the decane layer and undergoes significant surface denaturation [15]. Euston et al. [16] and Zhao & Cieplak [18] have determined the effect of reducing disulphide bonds on the conformation of LTP at the air-water interface. Euston et al. [16] using an all-atom MD model report that reduction of all four disulphide bonds leads to an increase in the conformational entropy of the protein which opposes the adsorption of the LTP. The coarse-grained model of LTP adsorption used by Zhao & Cieplak [18] contradict this and shows that fully reduced LTP adopts a flatter conformation at the interface than the non-reduced LTP. It should be noted that the timescales of the simulations in these two studies are very different. The all-atom simulation was limited to a few 10's of ns and this may not have been long enough for an extended conformation to form. It is possible that the increase in conformational entropy from removing the disulphide bonds increases the energy barrier to initial adsorption. The shorter all-atom simulations cannot overcome this barrier, but the coarse-grained simulations are run over a long enough timescale for extended conformations to occur. Once the LTP starts to spread at the air-water

interface, the extra surface-protein interactions are sufficient to compensate for the increased conformational entropy.

Zhao & Cieplak [18] have also used their coarse-grained model to compare the adsorption behaviour at both air-water and oil-water interfaces of five proteins of differing size, tryptophan cage (20 amino acids), streptococcal protein G (56 amino acids), hydrophobin HFBI (72 amino acids), LTP1 (91 amino acids) and hen egg-white lysozyme (129 amino acids). They found that all proteins spread at the interface, and that adsorption was stronger to the oil-water than the air-water interface. Diffusion of proteins across the surface was slower at the oil-water than air-water interface and there was higher probability of desorption form the air-water interface. Earlier simulations of LTP [15] and lattice proteins [19] had noted that the protein molecule penetrates further into an oil-phase than an air phase. This may explain the larger adsorption energy at the oil-interface and the slower diffusion. The more viscous oil phase would create a greater drag on the protein diffusional motion than would an air phase.

Surfactant proteins are a second group of proteins the adsorption behaviour of which has been widely simulated. These are highly surface-active proteins produced by living organisms and have specific surface chemical functions. The most widely known and studied are the hydrophobins. These are small, hydrophobic proteins expressed by filamentous fungi [20]. There function is to facilitate sporulation by reducing the surface tension at the water surface so that hyphae can push through the surface to release spores into the air. Two general types of hydrophobin have been identified, class I and class II. There is little sequence homology between hydrophobins apart from a set of 8 conserved cysteine residues that form 4 disulphide bonds and impart a high conformational stability [20]. Additionally, the distribution of hydrophobic amino acids

is similar within each class and leads to a hydrophobic patch that is important in surface adsorption [21]. Class II hydrophobins show a greater degree of sequence homology between the conserved cysteine residues than do class I hydrophobins [20].

Euston [22**] has simulated the self–association of the class II hydrophobin HFBI from *Trichoderma reesei*, showing that two molecules associate through interaction between their respective hydrophobic patches. Simulated adsorption of HFBI at air-water, decane-water and diphospatidylcholine (DPPC) bilayer-water interfaces allowed the role of the hydrophobic patch in adsorption to be elucidated [22**]. HFB1 adsorbs with the hydrophobic patch parallel to both the air-water and decane water interfaces, but normal to the DPPC interface (Figure 2), probably reflecting the presence of the hydrophilic phosphatidyl choline group at the interface with the water. The tertiary structure of HFBI is only slightly perturbed at the air-water surface, slightly more at the DPPC-water and substantially unfolded at the decane-water interface. Raffaini et al. [23] have confirmed the high conformational stability of HFBII at the air-water interface and the importance of the hydrophobic patch in the adsorption process. Cheung has used a coarse-grained model for the adsorption of HFBII and HFBI, another class II hydrophobin from *T. reesei*, at the octane-water interface [24] and has used this to show that HFBI and HFBII adsorb essentially irreversibly to the interface. The simulated free energy of adsorption is in the range 80-100 k_BT, which is considerably more than for most biomolecule surfactants.

Bacteria also produce surfactant proteins that are similar in function to the fungal hydrophobins. One of these, BslA, produced by *B. subtilis* has no sequence similarity with fungal hydrophobins, but does share the characteristic hydrophobic patch [25**]. The function of BslA is to coat the surface of bacterial biofilm, with the hydrophobic patch pointed outwards to form a protective, non-wetting hydrophobic interface.

Interestingly, BslA does not have the conserved 8 cysteine motif, which forms the four disulphide bonds that stabilize hydrophobins and allows the hydrophobic patch to remain on the outside of the molecule. This raises the question as to how the surface hydrophobic patch observed in the X-ray structure in BslA is stabilised in aqueous systems in the absence of the structural disulphides, a mechanism that allows BslA to be soluble and monomeric in solution, unlike hydrophobins. Bromley et al. [25**] have carried out experimental and coarse-grained MD simulations that have shown that in solution the hydrophobic patch amino acids adopt a random coil conformation that protects them from exposure to the aqueous environment. When BslA adsorbs to an interface, the hydrophobic patch amino acids insert into the non-aqueous phase, and refold into three beta sheets. This reforms the hydrophobic patch, and allows the BslA to self-associate at the interface through inter-molecular hydrophobic interactions forming a rigid stabilising adsorbed protein film. Evidence for this includes a small energy barrier to adsorption, elucidated through coarse-grained MD simulations, that suggests a conformation upon surface adsorption. Brandani et al. [26**] have extended the coarse-grained simulations on BslA and in doing so have highlighted the biotechnological potential of the molecule. The structure of BslA, where there is a clear partitioning between hydrophobic and hydrophilic ends of the molecule is reminiscent of amphiphilic Janus colloids. These are particles with distinct hydrophilic and hydrophobic faces. When Janus colloids adsorb to interfaces, they are known to be switchable, i.e. they can adopt conformations where the hydrophilic or hydrophobic regions are presented to the aqueous phase depending on environmental conditions. Brandani et al [26**] noted that the crystal structure of BslA [27] shows two main forms and that these adopt different conformations at the cyclohexane-water interface. One adsorbed conformation sees the BslA upright and presenting the hydrophobic patch

to the water, and the second tilts at the interface and exposes more of the hydrophilic part of the molecule to the oil phase. MD simulations revealed that the energy difference between the two conformations is relatively small, which suggests the possibility of BslA possessing a conformational switch that can be exploited to alter the conformation and interfacial properties of the BslA adsorbed film, although as the authors point out a practical solution to controlling switching will need to be found.

A further surfactant protein that undergoes an unsual conformational change upon adsorption is ranspumin-2 (rsn-2), the protein responsible for formation and stability of the foam nests of the túngara frog (*Engystomops pustulosus*) [28]. The structure of rsn-2 has no obvious amphiphilic structure, with the hydrophobic amino acids predominantly localised in a hydrophobic core. To explain the interfacial activity, this led Mackenzie et al. [29] to hypothesize what they termed a clam-shell transition for rsn-2 adsorbing to a hydrophobic interface, where two halves of the molecule opened up to expose the hydrophobic core to the interface. Somewhat surprisingly for such a significant change in the tertiary structure, the secondary structure is unaffected [29]. MD simulation has revealed a two-state adsorption mechanism [30, 31] at the air-water and cyclohexane-water interfaces. First, the flexible, random coil N-terminal end of the rsn-2, which contains a high proportion of hydrophobic amino acids, contacts the oil-surface and recruits the protein to the interface. This is followed by adsorption of the globular part of rsn-2 and conformational unfolding. This mechanism has been confirmed using variants of rsn-2. The influence of the N-terminal on adsorption was probed using two mutant proteins, one with the first three amino acids (Δ1-3), and a second with amino acids 1-15 (Δ1-15) from the N- terminal deleted. Similarly, two mutants at the C-terminal end (Δ96) and (Δ89-96) were constructed. A further variant was used, where two disulphide bonds are introduced to prevent the clam-shell

unhinging of the globular part of the protein. The N-terminal deletions slowed the kinetics of adsorption to the interface. This was also observed for the Δ89-96 C-terminal deletant under some conditions. These results confirmed the importance of the N-terminal amino acids in rsn-2 adsorption and also suggests that the C-terminal end plays a role, possibly in ensuring the correct orientation of rsn-2 for adsorption. Furthermore, the rsn-2 with additional cysteine bridges adsorbed as quickly as the native structure, but occupied a smaller area at the surface, again supporting the view that consolidation of the rsn-2 adsorption is through a clam-shell like unhinging at the surface [30, 31**].

3. Modelling of Protein Adsorption at Solid Surfaces

Early computational studies of protein adsorption at solid surfaces have been reviewed elsewhere [32-34], and here we concentrate on more recent studies over the past few years. The mechanism of adsorption and in particular the interactions involved and the driving force for adsorption will differ between a fluid and a solid interface. Hydrophobic interactions will predominate at uncharged fluid interfaces, whereas a combination of hydrophobic and electrostatic interactions is more important at charged surfaces.

Several simulation studies have demonstrated the importance of the adsorption driving force. Ding et al. [35**] have investigated serum protein adsorption on nanoparticle surface and its effect on cellular delivery of nanoparticle through dissipative particle dynamics (DPD) coarse-grained simulations. The negatively charged HSA did not adsorb spontaneously to hydrophilic or negatively charged nanoparticle surfaces, but could adsorb onto the charged and hydrophobic surface to form a protein corona. Furthermore, the protein corona changed the way in which hydrophobic and cationic nanoparticles interact with model cell membranes, and in particular enhanced the phagocytosis (movement through the membrane) of cationic nanoparticles.

The adsorption of proteins and peptides to uncharged surfaces will proceed independently of electrostatic interactions. In this case, Penna et al. [36] have demonstrated the importance of a layer of water with orientational order at the solid surface (one layer tightly bound to the surface, the second more loosely associated with the first layer). Adsorption of a small peptide was shown to proceed through three stages, diffusion of the peptide toward the surface; interaction of the peptide with the outer of the two surface bound water layers; and irreversible adsorption surface through adsorption of amino acid residues to the solid surface. The rate of diffusion to the interface for the peptide was found to faster than expected from simple diffusion. Penna et al. [36] attribute this to the orientational ordering of the water in the two layers adjacent to the surface. The orientation of the water dipole in the water layer is such that the surface acquires a charge, and long range electrostatic interactions between the peptide and surface bias the diffusion of the peptide towards the surface. Once the peptide reaches the ordered water layer adjacent to the surface polar groups on the peptide form hydrogen bonds with the first water layer, and reversibly anchor the peptide adjacent to the surface. This is followed by a slower insertion of the peptide into the more strongly bound inner water layer. Once the peptide has inserted into this layer it is more strongly bound to the interface and a gradual consolidation of surface binding occurs as further amino acid residues penetrate into the adsorbed water layer and contact the surface. Although Penna et al. [36] simulations were for a small peptide at a non-specific solid surface, there is no reason to expect that the same or similar mechanism does not contribute to the adsorption of larger proteins at uncharged and possibly charged interfaces.

The adsorption of peptides to silica nanoparticles offers a possible route to designing new drug delivery systems and thus has a great potential for future therapeutics.

Kubiak-Ossowska et al. [37] have followed how a spontaneous membrane-translocating peptide (SMTP), known to directly penetrate cell membranes, adsorbs to silica surfaces. MD simulation results showed the adsorbed SMTP flattens onto the interface irrespective of surface charge density (siloxide-rich surfaces with an electric field across the water/peptide space, hydroxylated silica surfaces with no electric field, and a mixture of sites and a weaker electric field to mimic silica nanoparticles). Screening of surface charge by addition of salt does not result in desorption, suggesting non-electrostatic forces also contribute to the final adsorbed conformation. In addition, they found that two polar residues in the peptide, Arg6 and Arg9, form favourable electrostatic interactions with surface, agreeing with several studies identifying arginine as an important residue for anchoring peptides to surfaces.

In another study, Kubiak-Ossowska et al. [38] probed the mechanism of adsorption of human fibronectin adhesion synergy region in repeat 9 ($FN^{III}9$) domain adsorption onto various model surfaces, finding that adsorption was rapid and driven by electrostatics. A similar conclusion was drawn when the same group simulated the adsorption of Hen Egg White Lysozyme (HEWL) on a silica surface [39]. The MD simulations indicated that at pH7 the adsorption is strong and rapid, and that the main adsorption driving force is electrostatics, supplemented by weaker hydrophobic forces. Lysozyme and bovine serum albumin (BSA) are popular model proteins for studying adsorption to various solid surfaces [40-45]. In particular, MD simulations have been employed to study how the negatively charged BSA adsorb to a model silica surface that is also negatively charged [40]. During the simulation, BSA rotates to present its IIB subdomain toward the silica surface, which orientates the negatively charged domains away from the surface. Positively charged Lys residues extend their side chains toward the surface, driven by electrostatic interaction between positively charged side

chains on Lys and the negatively charged surface. In this orientation, the side chains of Lys are able to penetrate both hydration water layers above the surface and form strong interactions with the surface, creating a stable adsorbed state [40]. The adsorption occurs despite the protein and surface both having the same net charge, supported by the effects of screening ions, the surface water layers and hydrophobic forces [41**]. Additionally, 8 H-bonds form between the protein and the surface hydration water layers, further strengthening adsorption and reducing the chance of desorption. The adsorbed BSA maintains its secondary and tertiary structure, so that its functionality may well be preserved.

In addition to silicon surfaces, carbon surface such as graphene-water or graphite-water, or carbon nanotube-water interfaces are now receiving increasing attention. Vilhena et al. [42] have used computational simulations to study the difference between free and forced adsorption of BSA on graphene. Free adsorption occurred with little structural rearrangements, and even if the adsorption was forced, they observed that the BSA was able to preserve the structural properties of the majority of its binding sites. In another study, Mücksch et al. [43] modelled the adsorption and forced desorption of BSA and lysozyme on a graphite surface, using MD simulations. Lysozyme retains much of its secondary structure during adsorption, whereas BSA loses it almost completely and also becomes almost fully unfolded during pull-off, in contrast to what is observed at the silicon dioxide surface. Lysozyme adsorption on different surfaces was also studied by Yu et al. [46**] with coarse-grained MD (CGMD) simulations. An important finding was that the conformation change of lysozyme on the hydrophobic surface was larger than on any other studied surfaces. Significant structural changes ensuing from the non-covalent absorption of bovine beta-lactoglobulin (BLG) on the hydrophobic surface of polystyrene nanoparticles was also

observed by Miriani et al. [47]. Simulation resulted in very quick induction of unfolding events, that were essentially complete within just 5 ns of simulated time.

The surface topography effects in protein adsorption on single-walled carbon nanotubes (SWNT) was studied by Raffaini et al. [48]. MD simulations showed that proteins favourably interact with the hydrophobic surfaces irrespective of their secondary structure. Moreover, the adsorption strength depends on the surface topography of SWNT; it is slightly weaker on the outer convex surfaces of SWNT, but enhanced on the inner concave surface, whilst being intermediate for flat graphene.

Several simulation studies have demonstrated also the importance of the orientation of proteins on their adsorption at surfaces. Liu et al. [49**] have studied the orientation of a prototype and mutated protein G B1 adsorbed on positively and negatively charged self-assembled monolayers by using Monte Carlo and all-atom MDs, considering that the orientation of an antibody plays a key role in the development of immunosensors. Simulation results showed that both proteins adsorb on charged surfaces with preferred orientations. Moreover, the mutant demonstrates narrower orientation distributions than does the prototype, which was caused by the stronger dipole of the mutant [49**]. In addition, protein adsorption was induced by the competition of electrostatic and vdW interactions, with the electrostatic interaction energy displaying distinctly higher values. Yu et al. [46**] have also found that the active sites of lysozyme face the hydrophobic surface with a “top end-on” orientation and they are exposed to the liquid phase on the hydrophilic surface with a “back-on” orientation.

The orientation and adsorption mechanism of candida antarctica lipase B (CalB), on four different nanomaterial surfaces has been explored by a combination of parallel tempering Monte Carlo (PTMC) and MD simulations by Zhao et al. [50*]. CalB is a

biocatalyst for hydrolysis and esterification that plays an important role in the production of biodiesel. They found that lipase adsorbs strongly onto the hydrophobic graphite surface by forming favourable π-π contacts and hydrophobic interactions, and the active site orients toward the solution. On the other hand, adsorption of lipase onto the hydrophilic TiO2 surface was weak because of the two strongly adhered water layers, allowing the lipase to reorient and desorb. On positively and negatively charged surfaces, the orientation distributions of lipase were narrow and opposite. For the positive surface, the interaction between CalB and the surface was weak, unstable and the protein adsorbed and then desorbed, without the protein's orientation changing much. The negative charge of lipase promoted its binding to the positive surface with a strong interaction, also with no change in the protein orientation. A similar computational approach has been used by this group to investigate the adsorption of hydrophobin (HFBI) on four different self-assembled monolayers [51**], finding that the orientation of HFBI adsorbed on neutral surfaces is dominated by a hydrophobic dipole. The hydrophobic patch of HFBI adsorbs on the hydrophobic surface by adopting a nearly vertical hydrophobic dipole relative to the surface, and it is nearly horizontal when adsorbed on the hydrophilic surface, while for the charged surfaces HFBI adopts a nearly vertical electric dipole (Figure 4). These results mirror those found for fluid interfaces by Euston [22**] where the hydrophobic patch is parallel to the surface (vertical dipole) at air- and decane-water interfaces, but normal to the interface (horizontal hydrophobic dipole) at the more hydrophilic DPPC-water surface. The unique structure and surface activity of hydrophobins has also led to the investigation of the adsorption of other hydrophobins at solid surfaces. Ley et al. [52] have employed all-atom MDs to study initial stages of the spontaneous adsorption of a monomeric class I hydrophobin EAS on fully hydroxylated silica. They found two

possible binding motifs for EAS hydrophobin; the unfolded Cys7-Cys8 loop is possible when hydrophobin adsorbs through residues 20–24 and 38–42 of the Cys3-Cys4 loop. In addition, the presence of areas void of water allows the penetration of hydrophobic side chains, and the interactions with the interfacial water layer allows the formation of both intermittent and long-lasting interactions with this layer, bringing the protein closer to the surface and promoting the protein surface adhesion.

Conclusions

The molecular simulation of protein adsorption at fluid and solid surfaces highlights a number of important differences between the two interfaces. The driving force for proteins to adsorb at fluid and solid surfaces differs between the interfaces. At air-water and oil-water surfaces, there is a lack of long-range electrostatic interactions and contact with the surface is through simple diffusion. Ordering of water close to solids surfaces leads to the water dipole allowing longer ranged electrostatic interactions between the partial charges of the water dipole and charged groups of the protein leading to biased diffusion of the protein to the surface. Similarly, contact between the protein and interface is controlled differently. At solid surface, interaction is initially between charged amino acid side-chains and the ordered hydration water layer, followed by electrostatic surface-protein interactions. At fluid interface, the principle interactions are hydrophobic and arise form unfolding of the protein and exposure of the hydrophobic core. With this insight, it may be possible to better select or design proteins to more efficiently form and stabilise interfaces in systems such as food emulsions and foams, or to promote adsorption to solid surface and conformational stability in medical devices.

Of particular interest are some of the unique mechanisms for adsorption and conformational change in surfactant proteins such as hydrophobin, BslA and rsn-2. With further computational work and insight, one day we may be able to design proteins so they mimic these mechanisms giving the possibility of producing interfacial stabilisers with controlled and tuned properties such as the switchable interfacial properties of BslA.

One word of caution should be attached to the simulation results discussed in this review, and which is of particular significance to fluid interface. All of these simulations have involved the adsorption of a single molecule at an interface, and ignore any effect other adsorbed proteins may have on the conformation. Lateral electrostatic interactions and steric interactions between adsorbed protein molecules will modify the adsorbed conformation. At liquid-liquid or gas-liquid interfaces electrostatic interactions play a more significant role than for solid-liquid interfaces because of the much lower dielectric permittivity and larger electrical double-layer thickness. As a result, the protein molecules interact with each other over longer distances, which influence not only conformational unfolding, but also can induce protein aggregation. The effect of these crowding effects in the protein adsorbed layer are ignored in most MD simulations of adsorbed proteins. In future, it would be informative to extend simulations to study such effects.

References

1. S. Damodaran, Protein Stabilization of Emulsions and Foams, Journal of Food Science 70 (2006) R54-R66.
2. E. Sadeghinezhad, S.N. Kazi, M. Dahari, M.R. Safaei, R. Sadri, A. Badarudin, A comprehensive review of milk fouling on heated surfaces, Crit Rev Food Sci Nutr 55 (2015) 1724-43.
3. E. Flechsig, I. Hegyi, M. Enari, P. Schwarz, J. Collinge, C. Weissmann, Transmission of scrapie by steel-surface-bound prions, Mol Med 7 (2001) 679-84.
4. C. Ozkan, J. Santini, H. Gao, G. Bao, Biomicroelectromechanical Systems (BioMEMS), MRS Symp. Proc.; Materials Research Society: Warrendale, PA 773 (2003)
5. J.M. Kauffmann, Biosensors in the pharmaceutical domain, Ann Pharm Fr 60 (2002) 28-37.
6. S.R. Euston, *Modelling and computer simulation of food structures*, in *Food Microstructures: Microscopy, Measurement and Modelling*, V. Morris and K. K.Groves, Editors. 2013, Woodhead Publishing: London. p. 336-385.
7. D. Zare, J.R. Allison, K.M. McGrath, Molecular Dynamics Simulation of beta-Lactoglobulin at Different Oil/Water Interfaces, Biomacromolecules 17 (2016) 1572-81.
8. D. Zare, K.M. McGrath, J.R. Allison, Deciphering beta-Lactoglobulin Interactions at an Oil-Water Interface: A Molecular Dynamics Study, Biomacromolecules 16 (2015) 1855-61.
9.* D.L. Cheung, Adsorption and conformations of lysozyme and alpha-lactalbumin at a water-octane interface, J Chem Phys 147 (2017) 195101. [Simulation of two structurally similar proteins that exhibit different unfolding behaviour at oil-water interfaces].
10.* D.L. Cheung, Conformations of Myoglobin-Derived Peptides at the Air-Water Interface, Langmuir 32 (2016) 4405-14. [A demosntration of the effect of conformational stability/flexibility on the conformations adopted by peptides at an oil-water interface].
11. S. Poon, A.E. Clarke, C.J. Schultz, Structure–Function Analysis of the Emulsifying and Interfacial Properties of Apomyoglobin and Derived Peptides, Journal of Colloid and Interface Science 213 (1999) 193-203.
12. S.A. Holt, D.J. McGillivray, S. Poon, J.W. White, Protein Deformation and Surfactancy at an Interface, The Journal of Physical Chemistry B 104 (2000) 7431-7438.
13. K. Lindorff-Larsen and J.R. Winther, Surprisingly high stability of barley lipid transfer protein, LTP1, towards denaturant, heat and proteases, FEBS Lett 488 (2001) 145-8.
14. L. Moelskov Bech, P. Vaag, B. Heinemann, K. Breddam. *Throughout the brewing process barley lipid transfer protein 1 (LTP1) is transformed into a more foam-promoting form*. in *In Proceedings of Congress-European Brewery Convention*. 1995. Oxford University Press.
15. S.R. Euston, P. Hughes, M.A. Naser, R.E. Westacott, Comparison of the adsorbed conformation of barley lipid transfer protein at the decane-water and vacuum-water interface: a molecular dynamics simulation, Biomacromolecules 9 (2008) 1443-53.
16. S.R. Euston, P. Hughes, M.A. Naser, R.E. Westacott, Molecular dynamics simulation of the cooperative adsorption of barley lipid transfer protein and cis-isocohumulone at the vacuum-water interface, Biomacromolecules 9 (2008) 3024-32.
17. S.R. Euston, Molecular dynamics simulation of protein adsorption at fluid interfaces: a comparison of all-atom and coarse-grained models, Biomacromolecules 11 (2010) 2781-7.
18. Y. Zhao and M. Cieplak, Proteins at the air-water interface in a lattice model, The Journal of Chemical Physics 148 (2018) 094704.
19. R.E. Anderson, V.S. Pande, C.J. Radke, Dynamic lattice Monte Carlo simulation of a model protein at an oil/water interface, J. Chem. Fys. 112 (2000) 9167–9185.

20. M.B. Linder, G.R. Szilvay, T. Nakari-Setala, M.E. Penttila, Hydrophobins: the protein-amphiphiles of filamentous fungi, FEMS Microbiol Rev 29 (2005) 877-96.
21. J.M. Kallio, N. Hakulinen, J.P. Kallio, M.H. Niemi, S. Karkkainen, J. Rouvinen, The contribution of polystyrene nanospheres towards the crystallization of proteins, PLoS One 4 (2009) e4198.
22.** S.R. Euston, Molecular simulation of adsorption of hydrophobin HFBI to the air–water, DPPC–water and decane–water interfaces, Food Hydrocolloids 42 (2014) 66-74. [HFBI is shown to adsorb with its hydrophobic patch parallel to the air-water and decane water interfaces, but perpendicular to the DPPC-water interface. This is attrbuted to the more hydrophilic character of a DPPC bilayer surface].
23. G. Raffaini, R. Milani, F. Ganazzoli, G. Resnati, P. Metrangolo, Atomistic simulation of hydrophobin HFBII conformation in aqueous and fluorous media and at the water/vacuum interface, J Mol Graph Model 63 (2016) 8-14.
24. D.L. Cheung, Molecular simulation of hydrophobin adsorption at an oil-water interface, Langmuir 28 (2012) 8730-6.
25.** K.M. Bromley, R.J. Morris, L. Hobley, G. Brandani, R.M. Gillespie, M. McCluskey, U. Zachariae, D. Marenduzzo, N.R. Stanley-Wall, C.E. MacPhee, Interfacial self-assembly of a bacterial hydrophobin, Proc Natl Acad Sci U S A 112 (2015) 5419-24. [MD simulation is used to explain how the hydrophobic patch in BslA is protected from the aqueous phase in solution, but refolds to allow adsorption at interfaces].
26.** G.B. Brandani, M. Schor, R. Morris, N. Stanley-Wall, C.E. MacPhee, D. Marenduzzo, U. Zachariae, The Bacterial Hydrophobin BslA is a Switchable Ellipsoidal Janus Nanocolloid, Langmuir 31 (2015) 11558-63. [The first time that the similarity between hydrophobin-like proteins and Janus collodis has been made. MD simualtion is used to demonstrate the mechanism for switching in a protein Janus particle].
27. L. Hobley, A. Ostrowski, F.V. Rao, K.M. Bromley, M. Porter, A.R. Prescott, C.E. MacPhee, D.M. van Aalten, N.R. Stanley-Wall, BslA is a self-assembling bacterial hydrophobin that coats the Bacillus subtilis biofilm, Proc Natl Acad Sci USA 110 (2013) 13600-5.
28. R.I. Fleming, C.D. Mackenzie, A. Cooper, M.W. Kennedy, Foam nest components of the tungara frog: a cocktail of proteins conferring physical and biological resilience, Proc Biol Sci 276 (2009) 1787-95.
29. C.D. Mackenzie, B.O. Smith, A. Meister, A. Blume, X. Zhao, J.R. Lu, M.W. Kennedy, A. Cooper, Ranaspumin-2: structure and function of a surfactant protein from the foam nests of a tropical frog, Biophys J 96 (2009) 4984-92.
30. R.J. Morris, G.B. Brandani, V. Desai, B.O. Smith, M. Schor, C.E. MacPhee, The Conformation of Interfacially Adsorbed Ranaspumin-2 Is an Arrested State on the Unfolding Pathway, Biophys J 111 (2016) 732-742.
31.** G.B. Brandani, S.J. Vance, M. Schor, A. Cooper, M.W. Kennedy, B.O. Smith, C.E. MacPhee, D.L. Cheung, Adsorption of the natural protein surfactant Rsn-2 onto liquid interfaces, Phys Chem Chem Phys 19 (2017) 8584-8594. [A very elegant but simple use of coarse-grained MD simulation to confirm the clam-shell unhinging mechanism for rsn-2 adsorption at an oil-water interface].
32. S.R. Euston, Computer simulation of proteins: adsorption, gelation and self-association, Current Opinion in Colloid & Interface Science 9 (2004) 321-327.
33. R.A. Latour, Molecular simulation of protein-surface interactions: benefits, problems, solutions, and future directions, Biointerphases 3 (2008) FC2-12.
34. M. Rabe, D. Verdes, S. Seeger, Understanding protein adsorption phenomena at solid surfaces, Adv Colloid Interface Sci 162 (2011) 87-106.
35.** H.M. Ding and Y.Q. Ma, Computer simulation of the role of protein corona in cellular delivery of nanoparticles, Biomaterials 35 (2014) 8703-10. [Dissipative particle dynamics simulations is used to demonstrate that the human serum albumin will adsorb onto mainly positively charged and hydrophobic nanoparticle surface].

36. M.J. Penna, M. Mijajlovic, M.J. Biggs, Molecular-level understanding of protein adsorption at the interface between water and a strongly interacting uncharged solid surface, J Am Chem Soc 136 (2014) 5323-31.
37. K. Kubiak-Ossowska, G. Burley, S.V. Patwardhan, P.A. Mulheran, Spontaneous membrane-translocating peptide adsorption at silica surfaces: a molecular dynamics study, J Phys Chem B 117 (2013) 14666-75.
38. K. Kubiak-Ossowska, P.A. Mulheran, W. Nowak, Fibronectin module FN(III)9 adsorption at contrasting solid model surfaces studied by atomistic molecular dynamics, J Phys Chem B 118 (2014) 9900-8.
39. K. Kubiak-Ossowska, M. Cwieka, A. Kaczynska, B. Jachimska, P.A. Mulheran, Lysozyme adsorption at a silica surface using simulation and experiment: effects of pH on protein layer structure, Phys Chem Chem Phys 17 (2015) 24070-7.
40. K. Kubiak-Ossowska, B. Jachimska, P.A. Mulheran, How Negatively Charged Proteins Adsorb to Negatively Charged Surfaces: A Molecular Dynamics Study of BSA Adsorption on Silica, J Phys Chem B 120 (2016) 10463−10468.
41.** K. Kubiak-Ossowska, K. Tokarczyk, B. Jachimska, P.A. Mulheran, Bovine Serum Albumin Adsorption at a Silica Surface Explored by Simulation and Experiment, J Phys Chem B 121 (2017) 3975-3986. [MD simulations is used to show how the negatively charged BSA at pH7 adsorbs to the negatively charged silica surface, revealing a unique orientation with preserved secondary and tertiary structure].
42. J.G. Vilhena, P. Rubio-Pereda, P. Vellosillo, P.A. Serena, R. Perez, Albumin (BSA) Adsorption over Graphene in Aqueous Environment: Influence of Orientation, Adsorption Protocol, and Solvent Treatment, Langmuir 32 (2016) 1742-1755.
43. C. Mücksch and H.M. Urbassek, Forced Desorption of Bovine Serum Albumin and Lysozyme from Graphite: Insights from Molecular Dynamics Simulation, The Journal of Physical Chemistry B 120 (2016) 7889-7895.
44. K. Tokarczyk, K. Kubiak-Ossowska, B. Jachimska, P.A. Mulheran, Energy Landscape of Negatively Charged BSA Adsorbed on a Negatively Charged Silica Surface, J Phys Chem B 122 (2018) 3744-3753.
45. L. Perez-Fuentes, C. Drummond, J. Faraudo, D. Bastos-Gonzalez, Adsorption of Milk Proteins (beta-Casein and beta-Lactoglobulin) and BSA onto Hydrophobic Surfaces, Materials 10 (2017) 893.
46.** G. Yu, J. Liu, J. Zhou, Mesoscopic coarse-grained simulations of lysozyme adsorption, J Phys Chem B 118 (2014) 4451-60. [A comprehensive study that adopts coarse-grained simulations to explore the adsorption behavior of lysozyme on hydrophobic, neutral hydrophilic, zwitterionic, negatively charged, and positively charged surfaces at the mesoscopic microsecond time scale].
47. M. Miriani, I. Eberini, S. Iametti, P. Ferranti, C. Sensi, F. Bonomi, Unfolding of beta-lactoglobulin on the surface of polystyrene nanoparticles: experimental and computational approaches, Proteins 82 (2014) 1272-82.
48. G. Raffaini and F. Ganazzoli, Surface topography effects in protein adsorption on nanostructured carbon allotropes, Langmuir 29 (2013) 4883-93.
49.** J. Liu, C. Liao, J. Zhou, Multiscale simulations of protein G B1 adsorbed on charged self-assembled monolayers, Langmuir 29 (2013) 11366-74. [A combination of parallel tempering Monte Carlo and all-atom MD simulations is used to show that the orientation of prototypical and mutated protein G B1 adsorbed on positively and negatively charged self-assembled monolayers].
50.* D. Zhao, C. Peng, J. Zhou, Lipase adsorption on different nanomaterials: a multi-scale simulation study, Phys Chem Chem Phys 17 (2015) 840-50. [A combination of parallel tempering Monte Carlo and all-atom MD simulations is used to understand the orientation and adsorption mechanism of candida antarctica lipase B on four different nanomaterial surfaces with different surface chemistry].
51.** C. Peng, J. Liu, D. Zhao, J. Zhou, Adsorption of hydrophobin on different self-assembled monolayers: the role of the hydrophobic dipole and the electric dipole, Langmuir 30 (2014) 11401-11. [Parallel tempering Monte Carlo and all-atom MD

simulations is used to explain the adsorptions of hydrophobin on four different self-assembled monolayers. This simulation study suggests that the hydrophobic dipole may be applied to predict the probable orientations of protein adsorbed on hydrophobic surfaces].

52. K. Ley, A. Christofferson, M. Penna, D. Winkler, S. Maclaughlin, I. Yarovsky, Surface-water Interface Induces Conformational Changes Critical for Protein Adsorption: Implications for Monolayer Formation of EAS Hydrophobin, Front Mol Biosci 2 (2015) 64.

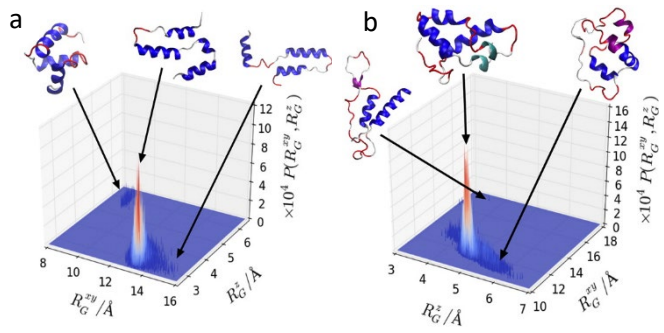

Figure 1 - Two-dimensional probability distribution for the most probable adsorbed conformations of (a) myoglobin peptide 1-55 and (b) peptide 56-113. The most probable conformation (highest peak in probability distribution) is extended for peptide 1-55 and compact for peptide 56-131. Reprinted with permission from Cheung, D.L., 2016. Conformations of Myoglobin-Derived Peptides at the Air–Water Interface. Langmuir, 32(18), 4405-4414. Copyright 2016 American Chemical Society.

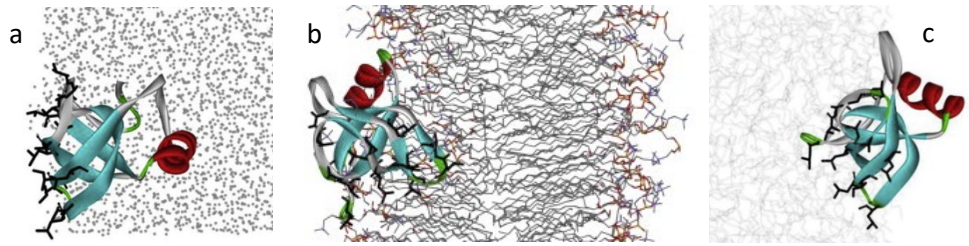

Figure 2 – Adsorbed conformations of hydrophobin HFBII at (a) vacuum-water, (b) DPPC-bilayer-water, (c) decane-water interfaces. The hydrophobic patch amino acids of HFBII are highlighted in black line representation. The hydrophobic patch aligns parallel to the vacuum-water and decane-water interface, but perpendicular to the DPPC-water interface. Reprinted from Food Hydrocolloids, 42, S.R.Euston, Molecular simulation of adsorption of hydrophobin HFBI to the air–water, DPPC–water and decane–water interfaces, 66-74. Copyright (2014), with permission from Elsevier.

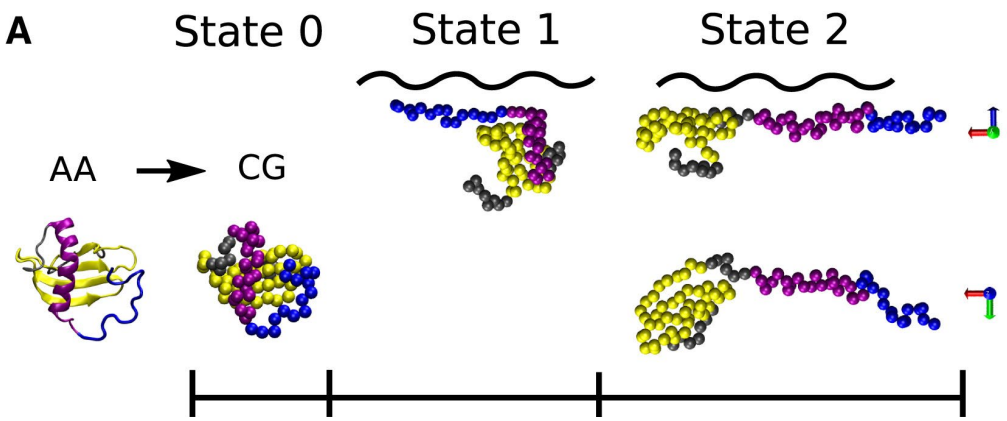

Figure 3 - Schematics of the coarse-graining (CG) from the all-atom NMR structure (AA), and typical rsn-2 configuration at different adsorption stages: in bulk (state 0, corresponding to the native structure); with the flexible N-terminal region adsorbed at the interface (state 1); and fully adsorbed and partially unfolded in side-view (top) and top-view (bottom) (state 2). The N-terminal tail is shown in blue, and the C-terminal tail (89–96) are in gray. The interface is represented by the black wavy line. For the coarse-grained structures only the Cα beads are shown and not the side chains. Reprinted from Biophysical Journal, 111(4), Morris, R.J., Brandani, G.B., Desai, V., Smith, B.O., Schor, M. and MacPhee, C.E., The conformation of interfacially adsorbed ranaspumin-2 is an arrested state on the unfolding pathway, 732-742. Copyright 2016, with permission from Elsevier.

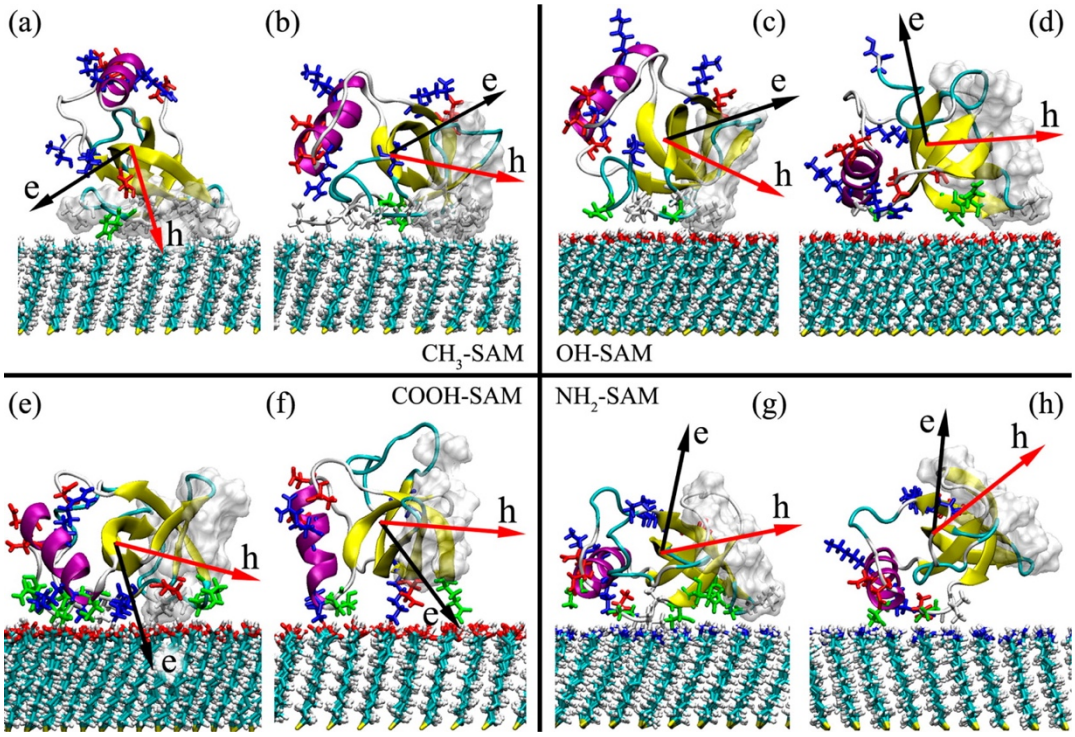

Figure 4 - Snapshots of HFBI adsorbed on four SAM surfaces from MD simulations: (a) from O1, O2, and O3 for HFBI on the CH3-SAM; (b) from O4 for HFBI on the CH3-SAM; (c) from O1 for HFBI on the OH-SAM; (d) from O3 and O4 for HFBI on the OH-SAM; (e) for HFBI on the 7.5% dissociated COOH-SAM; (f) for HFBI on the 15% dissociated COOH-SAM; (g) for HFBI on the 7.5% dissociated NH2-SAM; (h) for HFBI on the 15% dissociated NH2-SAM. The black and the red arrow represent the directions of the electric and hydrophobic dipole, respectively. The protein is represented in cartoon mode; the white and transparent area represents the hydrophobic patch. The positively charged residues are coloured in blue and the negatively charged residues are coloured in red. The SAM surfaces are shown in licorice mode. Residues within 3.5 Å from the surface are shown in licorice mode. Water and ions are not shown for clarity. Source: Reprinted with permission from Peng et al. [51**]. Copyright (2014) American Chemical Society.